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(54) **Method of and apparatus for measuring spectral absorption in opaque specimens and method of and apparatus for measuring microscopic absorption distribution**

Verfahren und Apparat zur Messung spektraler Absorption in undurchsichtigem Material und Verfahren und Apparat zur Messung einer Verteilung mikroskopischer Absorption

Méthode et appareil pour mesurer l'absorption spectrale dans des échantillons opaques et méthode et appareil pour mesurer une distribution d'absorption microscopique

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Description

The present invention relates to a method of and apparatus for measuring spectral absorption in scattering objects, e.g., suspensions or powders, and, more particularly, to a method of and apparatus for measuring spectral absorption characteristics of a component transmitted in a specific direction when a beam is applied to a specimen from a specific direction.

The present invention also relates to a method of and apparatus for measuring a microscopic absorption distribution of opaque speciments, e.g., biological specimens and, more particularly, to a method of and apparatus for measuring a microscopic absorption distribution, wherein unnecessary scattered light is removed to improve the resolution so that it is possible to accurately measure absorption in a very small region of a specimen.

Since the discovery of X-rays, techniques of externally observing the inside of a living body (e.g., human body) without damaging it (i.e., a bloodless or non-destructive measuring method) have been much required and developed in the field of biology, particularly in the medical field. These techniques employ gamma rays and X rays, which have the shortest wavelengths among the electromagnetic waves, and radio waves, which have the longest wavelengths among them. The technique that employs the former has already been put to practical use as X-ray CT, and the technique that employs the latter as NMR-CT (Magnetic Resonance Imaging, i.e., MRI).

On the other hand, fewer attempts have been made to apply spectroscopy that deals with the measurement and analysis of ultraviolet, visible, near infrared and infrared spectra, which is widely employed in the fields of physics and chemistry, to in vivo measurement. This is because biometry that employs light, particularly the one that utilizes the process of absorption or emission of light has many problems left unsolved in terms of "quantitativeness", which is the most basic matter. This is the reason why reproducibility is inferior and reliability is low in regard to the absolute values obtained in measurement that is conducted at the present time by using, for example, an apparatus that measures reflected spectra with a solid-state device, or a highly sensitive TV camera.

In a case where light is applied to a scattering object such as an organic tissue, if the light is received face to face at 180°, it is possible to take out rectilinearly propagating light to some extent. However, the spatial resolving power is not very high in the present state of art.

The difference in the spatial resolving power between X-rays and light cannot be made up in the present state of art. However, employment of light rays, particularly near infrared rays will enable imaging of a tissue oxygen concentration from the hemoglobin in the blood. These light rays will give information which is different from that obtained by other techniques such as NMR-CT and X-ray CT.

As for relatively thin tissues with a thickness of 3 to 5cm, it is possible to detect light transmitted thereby. This means that "photo-roentgenography" can be used for diagnostic purposes. Women's breasts have relatively homogeneous tissues and hence readily transmit light, and it is easy to detect the light transmitted thereby (thickness: up to about 3cm) owing to their configuration. For this reason, "photo-roentgenography" has been employed for a medical examination for breast cancer for a long time under the name of "diaphanography (lightscanning)".

Under these circumstances, the present inventor disclosed that a plane wave mixed in scattered light can be separated therefrom for observation by detecting only the 0-order spectrum (corresponding to the first dark ring of an Airy's disk) of the Fraunhofer diffraction image (Airy's disk) of the plane wave and, by so doing, most of the scattered light can be removed. See, for example, Japanese Patent Applications Nos. 01-62898 (1989), 01-250034 (1989) and 02-77690 (1990). More specifically, when only a 0-order Fraunhofer diffraction pattern of a plane wave as signal light is to be detected, the degree of separation of incoherent scattered light from the plane wave is given by (scattering intensity)/(transmitted plane wave intensity) $\fallingdotseq(\lambda/Dr)^2$

In other words, the larger the beam diameter or the entrance aperture diameter Dr of a highly directional detecting system, e.g., a heterodyne light-receiving system, a Michelson light-receiving system, a highly directional optical system, etc., in comparison to the wavelength λ, the more the scattered light attenuates, and the more the scattered light can be separated from the plane wave. As one example of a highly directional optical system used to realize such observation, the present inventor proposed an optical system comprising two pinholes P_1 and P_2 which are spaced apart from each other, as shown in Fig. 7. This optical system is arranged such that 0-order light is detected by a detector 23 through the pinhole P_2. The present inventor also proposed a highly directional optical system comprising a hollow, straight, long and thin glass fiber 35 the inner wall surface of which is coated with a light absorbing material, e.g., carbon, as shown in Fig. 8. Further, the present inventor proposed various highly directional optical systems such as those shown in Figs. 9 to 16: a highly directional optical system (Fig. 9) comprising an objective lens Ob and a pinhole P that is disposed on the focal plane thereof to pass only a 0-order Fraunhofer diffraction pattern formed by the obiective lens Ob; a highly directional optical system (Fig. 10) comprising a graded-index lens GL and a pinhole P (similar to that shown in Fig. 9) that is disposed on the focal plane at one end of the graded-index lens GL; a highly directional optical system (Figs. 11 and 12) in which the pinhole P is replaced with an optical fiber SM that functions in the same way as the pinhole P; a highly directional optical system (Figs. 12 and 15) in which an objective lens Ob2

which is similar to an objective lens Ob1 at the entrance side is disposed at the exit side of the pinhole P or the optical fiber SM in the above-described highly directional optical systems; and a highly directional optical system (Figs. 14 and 16) in which a graded-index lens GL2 which is similar to a graded-index lens GL1 at the entrance side is disposed at the exit side of the pinhole P or the optical fiber SM.

Incidentally, there are known methods of measuring absorption in opaque specimens, for example, the opal glass method in which a rectilinear component and a transmission and scattering component of a specimen that causes scattering are uniformly scattered by use of opal glass to measure a transmission integral extinction of the specimen [see, for example, Kazuo Shibata "Photobiology Series: Introduction to Spectral Measurement", pp.62-82 (June 20, 1976, Kyoritsu Shuppan K. K.)]. Heterogereous systems such as suspensions of particles, for example, cells, granules or solid power, absorb and scatter light, in general. Accordingly, it is difficult to obtain only absorption wavelength characteristics of such heterogeneous systems. For this reason, it is conventional practice to obtain a quantity with which real absorption wavelength characteristics can be approximated. More specifically, a transmission integral extinction is obtained to replace absorption. The transmission integral extinction is the cologarithm of the ratio of a bundle of light rays attenuated by both absorption and scattering to the incident light rays, which is not coincident with absorption characteristics, in general. In order to enable the transmission integral extinction to be approximated to real absorption characteristics as much as possible, if parallel transmitted rays and scatteringly transmitted rays are detected at the same capturing rate by a detector, the effect of scattering on the ratio of the light rays becomes small. As a method for this purpose, the opal glass method has been put to practical use. In addition, the transmission integrating sphere method, photoelectric surface contact method, etc. have been put to practical use as methods of minimizing the effect of scattering by capturing the entire transmitted rays comprising parallel transmitted rays and scatteringly transmitted rays. A method that utilizes both the contact and scattering methods jointly has also been employed as an intermediate method between the detection of parallel transmitted light rays and scatteringly transmitted light rays at the same capturing rate and the detection of the entire transmitted light rays.

Meantime, a measuring method such as that shown in Fig. 17 has heretofore been employed to measure a microscopic absorption distribution in a specimen that causes scattering. More specifically, light from a light source that emits light over a wide spectral range is passed through an interferometer for Fourier spectroscopy and then sent to either a transmission optical path or a reflection optical path through a transmission/reflection switching mirror. If the transmission optical path is selected, the illuminating light is condensed to a very small point on a specimen placed on a specimen table through a lower Cassegrain system that functions as a condenser lens. Light that is transmitted through the measuring point and light that is forwardly scattered at the measuring point are focused on an aperture through an upper Cassegrain system that functions as an objective lens, and the light that passes through the aperture is made incident on a detector to measure absorption characteristics at the measuring point. Thus, it is possible to measure a transmission microscopic absorption distribution in the specimen by repeating measurement similar to the above with the specimen table being scanned in directions X and Y. If the switching mirror is changed over to the reflection optical path, the illuminating light is condensed to a point on the specimen through the upper Cassegrain system, and light that is reflected and scattered backwardly from the measuring point is focused on the aperture through the same upper Cassegrain system. Thus, a reflection microscopic absorption distribution in the specimen can be measured in the same way as the above.

Fig. 18 shows another conventional microspectroscopic method that employs a combination of an optical microscope and a spectrophotometer to observe an absorption spectrum of a very small region. Light from a light source ℓ is formed into monochromatic light through a spectroscope m_0 to illuminate a diaphragm (pinhole) p. With the diaphragm p defined as a light source of a microscope system, light is passed through an illuminating microscope m_1. In consequence, a reduced image of the diaphragm p is formed on a specimen plane s. This image is enlarged through another microscope m_2 and led to a detector d. If a specimen is placed at the position s where the reduced image of the diaphragm is formed, it is possible to measure absorbance of only a local region in the specimen.

Incidentally, if the same measuring method that is used for a specimen that causes no scattering is employed to obtain an absorption spectrum of a specimen that causes scattering, the effect of scattering is large, so that it is impossible to obtain an accurate absorption spectrum. As techniques of measuring absorption in opaque specimens, methods wherein a transmission integral extinction is measured by use of opal glass or an integrating sphere, are known, as described above.

The above-described conventional methods, however, suffer from problems stated below: (1) the opal glass method involves the disadvantage that the light scattering power undesirably changes in accordance with wavelength; (2) the transmission integrating sphere method involves the disadvantage that a white reflecting material in the integrating sphere greatly decreases in reflectivity at a short wavelength, particularly near the ultraviolet region, even in the case of MgO powder, which is known as the best reflecting material, so that no reliable data can be obtained; (3) the photoelectric surface contact method that employs two detectors involves difficulty in obtaining the same wavelength sen-

sitivity characteristics, whereas the photoelectric surface contact method that employs a single detector involves difficulty in installing a specimen and a control within a limited space; and (4) the method that employs both the contact and scattering methods jointly suffers from the problem that it is necessary to properly select the size of a specimen and the distance and size of the detector, although it is superior to the former three methods.

In addition, the four conventional methods involve the common disadvantage that there are cases where a transmission integral extinction measured cannot be approximated to the absorption wavelength of suspended particles. More specifically, as the intensity of reflected rays increases, it becomes impossible to make approximation. When the scattering spatial pattern that is formed by a specimen depends upon wavelength, the wavelength change of scatteringly transmitted rays becomes different from that of the scatteringly reflected rays, so that no approximation can be made. In addition, it is difficult to perform absorption measurement that provides spatial resolving power such as specifies a location of absorption. Although the conventional methods are usable for measurement of absorption in sparse heterogeneous systems such as dilute suspensions, these methods cannot be applied to measurement of absorption in dense translucent objects such as biological specimens when scattering is so strong that the relationship of Kubelta-Munk is valid.

The microspectroscopic measuring method for infrared region that employs a Fourier spectroscope and the microspectroscopic measuring method for visible region that employs a diffraction grating spectroscope, which have been described above, have no measures taken to deal with opaque specimens that cause scattering and therefore involve large errors in the measurement of such specimens, so that no reliable data can be obtained. In other words, since unnecessary scattered light mixes in from the surroundings including the front and rear of the measuring point, it is impossible to measure accurate absorption characteristics. In addition, since Fraunhofer diffraction patterns other than the 0-order diffraction pattern enter the objective lens, the resolution is limited. The measuring method that is a simple combination of the measuring method employing opal glass or an integrating sphere, developed as a method of measuring absorption in opaque specimens, and the microspectroscopic measuring method involves the problem that the detected signal light is weak so that it is difficult to effect measurement, and it cannot therefore be put to practical use. Even if the detection sensitivy is improved markedly, since the method that employs opal glass or an integrating sphere is merely an approximation method, it cannot be used for a specimen for which approximation cannot be made when the intensity of reflected rays is high or when the wavelength change of scatteringly transmitted rays is the same as that of scattering reflected rays, due to large errors. Even if such a measuring -method can be used, the spatial resolving power is reduced. Thus, there is not yet a proper measuring method for an absorption spectrum in a very small region of an opaque specimen that causes scattering.

There is known, see GB-A 2191855 an apparatus and method having the features of the preamble of Claims 1 and 4. Such arrangement is intended to use the known characteristics of a Michelson interferometer to assist in detecting reflection sites in an integrated optic chip. The arrangement is not concerned, however, with the problem of detecting spectral absorption characteristics of an opaque light transmitting specimen.

Thus, it is a first object of the present invention to provide a method of an apparatus for measuring spectral absorption characteristics by removing scattered light rays in a scattering object, for example, a suspension or organic tissue, as much as possible and capturing parallel rays of a component transmitted in a specific direction (i.e., rectilinear component rays).

It is a second object of the present invention to provide a method of and an apparatus for measuring a microscopic absorption distribution, wherein unnecessary scattered light is removed to improve the resolution so that it is possible to accurately measure absorption in a very small region of an opaque specimen, for example, an organic tissue.

These objects are achieved by the characterising features of Claims 1 and 4.

Further preferred features and advantages of the invention will become apparent from the following description and the subordinate Claims, taken in conjunction with the accompanying drawings, in which:

Figure 1 is a view to explain the arrangement and operation of a Michelson light-receiving system as a highly directional detecting system which is utilised in the present invention;

Figure 2 shows an arrangement of an embodiment of the spectral absorption measuring apparatus employing a Michelson light-receiving system according to the present invention, which is applied to a transmitting specimen;

Figure 3 shows a specific example of apparatus for measuring spectral absorption characteristics in a microsize region of a specimen;

Figure 4 is a view for explanation of the arrangement and operation of a high-resolution detecting system employing a Michelson light-receiving system, which is employed in the method of and apparatus for measuring a microscopic absorption distribution in an opaque specimen according to the present invention;

Figure 5 shows the arrangement of one embodiment of the microscopic absorption distribution

measuring apparatus employing a Michelson light-receiving system according to the present invention, which is applied to a transmitting specimen;

Figure 6 shows a specific example of apparatus for measuring microscopic absorption distribution characteristics of a specimen according to the present invention;

Figures 7 to 16 show the arrangement of highly directional optical systems proposed by the present inventor prior to this application;

Figure 17 shows the arrangement of a conventional apparatus for measuring microscopic absorption distribution, which employs a Fourier spectroscope; and

Figure 18 shows the arrangement of a conventional apparatus for measuring microscopic absorption distribution in a very small specimen, which employs a diffraction grating spectroscope.

A Michelson light-receiving system is well known as a means capable of detecting a very small change in the refractive index or the like. In a Michelson light-receiving system 4, as shown in Fig.1, light from a laser 1 is split by a beam splitter BS into two light beams, one of which is reflected from mirrors M1 and M2 while being transmitted through a specimen S inserted in the path of the reflected light, and the transmitted light is combined with rectilinear light (described later) by a half-mirror HM. The rectilinear light (hereinafter referred to as "reference light") that is transmitted through the beam splitter BS passes through the half-mirror HM and strikes upon a moving mirror M that is moved as shown by the double-headed arrow in the figure. The light is reflected in the reverse direction and combined by the half-mirror HM with the light transmitted through the specimen S, and the resulting composite light is photoelectrically converted in a detector 2. The detector 2 outputs a signal upon which is superposed an interference signal the frequency of which corresponds to the speed of the moving mirror M. The intensity of the AC component of the output signal is proportional to the transmittance of the specimen S, and the phase of the signal depends upon the thickness or refractive index of the specimen S. The Michelson light-receiving system 4 is also capable of detecting a very small change in the refractive index or the like.

In addition, since the light component that is scattered by the specimen S in a direction different from the direction of the reference light does not overlap with the reference light on the detecting surface of the detector 2, no beat signal is generated thereby and the scattered light is detected as merely a DC component. Thus, the Michelson light-receiving system 4 also functions as a highly directional detecting system which is capable of readily removing such a scattering component and detecting only a light component that travels in the same direction as the reference light as well as detecting a weak signal as described above. Accordingly, the present invention makes use of the nature of the Michelson light-receiving system 3 as a highly directional detecting system.

Incidentally, the Michelson light-receiving system 4 detects the intensity of the light transmitted or scattered by the specimen S on the basis of a principle that will be briefly explained below. Assuming that the reference light which is to be combined is V_2 and the light transmitted or scattered by the specimen S (hereinafter referred to as "specimen light" in some cases) is V_1, these two light waves are expressed as follows:

$$V_1=A_1 \exp[-i(\omega_1 t+\phi_1)]$$

$$V_2=A_2 \exp[-i(\omega_2 t+\phi_2)]$$

When these two light waves V_1 and V_2 are observed (detected) in a superposed state, the detected signal S is given by

$$S=|V_1+V_2|^2=V_1 \cdot V_1^* + V_2 \cdot V_2^* + V_1 \cdot V_2^* + V_1^* \cdot V_2$$

Because

$$V_1 \cdot V_1^* = A_1^2, \quad V_2 \cdot V_2^* = A_2^2$$

and

$$V_1 \cdot V_2^* = A_1 A_2 \exp[-i(\omega_1-\omega_2)t - i(\phi_1-\phi_2)]$$

$$V_1^* \cdot V_2 = A_1 A_2 \exp[+i(\omega_1-\omega_2)t + i(\phi_1-\phi_2)]$$

$$V_1 \cdot V_2^* + V_1^* \cdot V_2 = 2A_1 A_2 \cos[(\omega_1-\omega_2)t+(\phi_1-\phi_2)]$$

the detected signal S is given by

$$S=A_1^2+A_2^2+2A_1 A_2 \cos[(\omega_1-\omega_2)t+(\phi_1-\phi_2)]$$

Since $\omega_1=\omega_2$ and $\phi_2=\phi_1+kt$ in the Michelson light-receiving sytem 4, the detected signal S is given by

$$S=A_1^2+A_2^2+2A_1 A_2 \cos kt$$

Thus, it is possible to obtain the amplitude A_1 of the specimen light V_1 from the size of the AC component of the detected signal.

Opaque specimens which may be subjected to spectral absorption measurement in the present invention are not those which block incident light completely and do not transmit it forwardly, but sparse heterogeneous systems such as dilute suspensions, e.g., biological specimens, and also specimens such as dense translucent biological specimens, in which substantially no light is transmitted directly therethrough without being scattered, but light that is multiple-scattered forwardly by scattering fine particles in the specimen emerges therefrom. It is a matter of course that a specimen which transmits light directly therethrough can be employed as an object of the measurement.

One embodiment of the method of and apparatus

for measuring spectral absorption characteristics of an opaque specimen according to the present invention will be described below.

Fig. 2 shows an apparatus to which the Michelson light-receiving system 4 shown in Fig. 1 is applied to measure spectral absorption characteristics of a transmitting specimen 20. In this arrangement, light that is emitted from a variable wavelength laser 10 is converted into a beam of parallel rays having a suitable diameter through a beam converter 11 and is then split by a beam splitter BS into two light beams, that is, rectilinear light and reflected light. A transmitting specimen 20 is inserted in the path of the reflected light that is passed via mirrors M1 and M2, and the light that is transmitted through the specimen 20 while being scattered is combined with the reference light by a half-mirror HM. The reference light that is transmitted through the beam splitter BS is further transmitted through the half-mirror HM to strike upon a moving mirror M that is moved as shown by the double-head arrow in the figure. The reference light that is reflected from the mirror M in the reverse direction is combined with the specimen light by the half-mirror HM, and the resulting composite light is photoelectrically converted in a detector 2, from which is obtained a signal having an interference signal superposed thereon, the frequency of the interference signal corresponding to the speed of the moving mirror M. Since the intensity of the AC component of the signal output from the detector 2 is proportional to the intensity of the light scattered in the transmitting specimen 20, spectral absorption characteristics can be obtained from the intensity of the AC component by sweeping the wavelength of the variable wavelength laser 10.

Fig.3 shows an apparatus that employs the Michelson light-receiving system 4 shown in Fig.2 Since this apparatus is modified simply by arranging the corresponding apparatus shown in Fig.2 into a vertical form, no special explanation will be needed. It should be noted that in Fig. 3 a driving system 14 is provided to move a moving mirror M along an optical axis.

The following is a description of embodiments of the method of and apparatus for measuring a microscopic absorption distribution in an opaque specimen according to the present invention.

The relationship between the specimen S and the beam of parallel rays in the highly directional optical systems 4 as shown in Figs. 2 and 3 is changed to that in high-resolution detecting system 40 arranged as shown in Fig. 4 thereby applying incident light to a very small point region that corresponds to a 0-order diffraction component of a Fraunhofer diffraction image formed by a lens, and thus making it possible to detect scattered light from only the very small point. More specifically, a condenser lens L1 with a relatively large numerical aperture (NA) is interposed at the light entrance side of the specimen S such that the back focal point of the lens L1 is coincident with a measuring point on the specimen S, and an objective lens L2 with a relatively large numerical aperture (NA) is disposed such that the front focal point of the lens L2 is coincident with the back focal point of the condenser lens L1. With this arrangement, light from a laser 1 is applied to a very small point on the specimen S through the condenser lens L1, and light emerging from the very small point is converted through the objective lens L2 into parallel rays traveling in a predetermined direction. Thus, only the light traveling in this direction is detected by a detector 2 on the principle of the highly directional detecting system 4. In this way, it is possible to detect scattered light only from a very small region corresponding to a 0-order diffraction component of a Fraunhofer diffraction image of the specimen S formed by a lens. Accordingly, employment of the above-described high-resolution detecting system 40 makes it possible to avoid mixing of unnecessary scattered light from the surroundings including the front and rear of the measuring point and also enables absorption characteristics of the specimen S to be measured with extremely high resolution.

Fig. 5 shows an apparatus which employs the high-resolution detecting system 40 comprising a Michelson light-receiving system, shown in Fig. 4, to measure a microscopic absorption distribution in a transmitting specimen 20. In this apparatus, light that is emitted from a variable wavelength laser 10 is converted through a beam converter 11 into a beam of parallel rays with a proprer diameter and then divided through a beam splitter BS into two light beams, that is, rectilinear light, i.e., reference light, and reflected light. A condenser lens L1 and an objective lens L2 are disposed confocally in the path of the reflected light that is passed via mirrors M1 and M2, and a transmitting specimen 20 is inserted at the confocal position. The light that is transmitted through the specimen 20 while being scattered is combined with the reference light by a half-mirror HM. The reference light, which is light passing through the beam splitter BS, passes through the half-mirror HM to strike upon a moving mirror M that is moved as shown by the double-headed arrow in the figure. The reference light, which is reflected from the moving mirror M in the reverse direction, is combined with the specimen light by the half-mirror HM, and the resulting composite light is photoelectrically converted in a detector 2. The detector 2 outputs a signal upon which is superposed an interference signal the frequency of which corresponds to the speed of the moving mirror M. Since the intensity of the AC component of the output signal is proportional to the intensity of the scattered light from the transmitting specimen 20, an absorption distribution in the specimen 20 can be measured by obtaining the size of the AC component at each measuring point while scanning the specimen 20 by an XY scanning device XY. It is also possible to measure a spectral absorption distribution by obtaining an absorption distribution while sweeping the wavelength of the variable wavelength laser 10.

Fig.6 shows an apparatus that employs the high-resolution detecting system 40 comprising a Michelson

light-receiving system, shown in Fig. 4 . Since this apparatus is a modification, made simply by arranging the corresponding apparatus shown in Fig 4 into a vertical form, no special explanation will be needed. It should be noted that in Fig. 16 driving system 17 is provided to move a moving mirror M along an optical axis.

Although in the foregoing embodiments the variable wavelength laser 10 is assumed to be oscillating continuously, it should be noted that a variable wavelength pulsed laser may also be employed. It is particularly preferable to employ a variable wavelength pulsed laser in the case of a specimen whose properties change rapidly when laser light is applied thereto continuously. Although no special explanation has been made on the detector 2, any known detecting means may be employed. The reflecting mirror in the Michelson interferometer may be either moved at a constant speed or oscillated by saw-tooth wave.

As has been described above, in the method of and apparatus for measuring spectral absorption in an opaque specimen according to one aspect of the present invention, a scattering specimen is illuminated with highly directional light of variable wavelength from a specific direction, thereby removing scattered rays as much as possible, and thus detecting the intensity of only parallel rays of a component transmitted in a specific direction (i.e., rectilinear component rays) by use of a highly directional Michelson light-receiving system.

It is therefore possible to measure spectral absorption characteristics of a scattering specimen with high accuracy without picking up scattered light in other undesired directions nor other noise light. In addition, the measurement of the control is exceedingly simplified in comparison to the conventional method and thus the measurement is extremely facilitated. Thus, the method and apparatus of the present invention are suitable for measuring spectral absorption of a component transmitted in a specific direction in not only sparse heterogeneous systems having spatial resolving power, for example, suspensions or organic tissues, but also dense transparent objects that cause scattering to a substantial degree.

In the method of and apparatus for measuring a microscopic absorption distribution in an opaque specimen according to another aspect of the present invention, a very small measuring point on a specimen is illuminated with a condensed light of high directivity, and light that diverges from the measuring point is converted into parallel rays, or left as it is in the form of a spherical wave, and then detected by use of a highly directional Michelson light-receiving system.

It is therefore possible to measure absorption in a very small region of a specimen with high resolution without picking up scattered light from the surroundings of the measuring point nor other noise light. Thus, the method and apparatus of the present invention are suitable for measurement of a microscopic absorption distribution in an opaque specimen, for example, an organic tissue.

Claims

1. An apparatus for measuring optical characteristics of a light scattering specimen (20,21) comprising a monochromatic light source (10) of variable wavelength; beam splitting means (BS) for dividing the light beam from said light source into first and second light beams; means (M) disposed in the path of said first light beam for varying the length of the optical path of said first light beam; means for supporting said light scattering specimen in the optical path of said second light beam; means (HM) for combining light from said second light beam propagated by said light scattering specimen (20,21) with light of said first light beam received from said varying means (M) and for projecting the resulting composite light in the same direction; and means (2) for receiving said composite light from said combining means (HM) and for converting said composite light into an electrical signal, said converting means including means for detecting the intensity of an AC component of said electrical signal; characterised in that said combining means (HM) is so arranged as to receive light from said second light beam propagated through an opaque specimen (20,21) held by said supporting means, that said varying means is arranged to vary the length of the optical path of said first light beam at a predermined speed that is so related to a narrow bandwidth of said monochromatic light source (10) that said AC component of said electrical signal has a frequency determined by interference between said first and second light beams whereby said means for detecting is arranged such that the intensity of said AC component is determined by the intensity of the second light beam propagated through said specimen and spectral absorption in said opaque specimen can be measured by variation of the wavelength of the emitted light.

2. An apparatus according to Claim 1, further comprising means for detecting the intensity only of said first light beam to provide a reference intensity and means for comparing said reference intensity with the intensity of said AC component to determine a transmission integral extinction and thus to obtain an absorption spectrum of said opaque specimen with variation of the wavelength of said monochromatic light source.

3. An apparatus as claimed in Claim 1 or 2, further comprising
a confocal optical system including two convergent optical systems (L1,L2) disposed in the path of said second light beam, said means for supporting said

light scattering specimen being arranged to dispose said specimen at the common focal point of said optical systems (L1,L2) and including means (XY) for scanning said specimen relatively to said focal point, whereby the microscopic absorption distribution of said specimen can be determined.

4. A method for measuring optical characteristics of a light scattering specimen, comprising the steps of: dividing the light beam from a monochromatic light source of variable wavelength into first and second light beams; disposing the said specimen in the path of said second light beam; combining light of said second light beam and propagated by said specimen with light of said first light beam, whilst varying the length of the optical path of said light of said first light beam; converting said combined light into an electrical signal and detecting the intensity of an interference component of said electrical signal; characterised in that said light of said second light beam combined with said light of said first light beam is light transmitted through said specimen; that the length of the optical path of said first light beam is varied at a speed so related to a narrow bandwidth of said light source that said interference component has an AC frequency the amplitude of which is determined by the intensity of the second light beam propagated through said specimen; that the wavelength of light emitted by said light source is varied and that corresponding variation of the amplitude of said interference component is detected to provide a measure of the spectral absorption of said specimen.

5. A method as claimed in Claim 4, wherein light of said second beam to be transmitted through said specimen is focussed to a point within the said specimen, said specimen is scanned relatively to the focal point, and only light transmitted from said focal point is combined with light of said first light beam, whereby the microscopic spectral absorption of said specimen is determined.

Patentansprüche

1. Vorrichtung zur Messung optischer Eigenschaften einer Licht streuenden Probe (20, 21), umfassend eine monochromatische Lichtquelle (10) von variabler Wellenlänge; einen Strahlteiler (BS) zur Aufteilung des Lichtstrahls der besagten Lichtquelle in einen ersten und einen zweiten Lichtstrahl; ein in dem Pfad des ersten Lichtstrahls angeordnetes Mittel (M) zur verstellung der Länge des optischen Pfads des ersten Lichtstrahls; ein Mittel zum Unterstützen der streuenden Probe in dem optischen Pfad des zweiten Lichtstrahls; ein Mittel (HM) zur Zusammenführung des Lichts des zweiten Lichtstrahls, welches von der streuenden Probe (20, 21) ausgeht, mit dem Licht des ersten Lichtstrahls, welches von dem Verstellungsmittel (M) empfangen wird, und zur Projektion des resultierenden, zusammengesetzten Lichts in die selbe Richtung; und ein Mittel (2) zum Empfang des zusammengesetzten Lichts von dem Zusammenführungsmittel (HM) und zur Umwandlung des zusammengesetzten Lichts in ein elektrisches Signal, wobei das Mittel zur Umwandlung ein Element zur Bestimmung der Intensität des Wechselanteils des elektrischen Signals aufweist, **dadurch gekennzeichnet,** daß das Zusammenführungsmittel (HM) derart angeordnet ist, um das Licht des zweiten Lichtstrahls aufzufangen, welches durch die undurchsichtige Probe (20, 21) hindurchläuft, die von dem Unterstützungsmittel gehalten wird; daß das Verstellungsmittel derart ausgebildet ist, um die Länge des optischen Pfads des ersten Lichtstrahls mit einer vorgegebenen Geschwindigkeit zu verstellen, die zu der engen Bandweite der monochromatischen Lichtquelle (10) derart in Beziehung gesetzt ist, daß die Wechselkomponente des elektrischen Signals eine Frequenz hat, die durch die Interferenz zwischen dem ersten und dem zweiten Lichtstrahl bestimmt ist; wobei das Mittel zur Bestimmung derart angeordnet ist; daß die Intensität der Wechselkomponente durch die Intensität des zweiten Lichtstrahls bestimmt wird, der sich durch die Probe ausbreitet; und die spektrale Absorption in der undurchsichtigen Probe kann durch Variation der Wellenlänge des ausgesendeten Lichts gemessen werden.

2. Vorrichtung nach Anspruch 1, weiterhin gekennzeichnet durch ein Mittel zur Bestimmung der Intensität ausschließlich des ersten Lichtstrahls, um eine Referenzintensität zu liefern, und durch ein Mittel zum Vergleich der Referenzintensität mit der Intensität der Wechselkomponente, um die Auslöschung der gesamten Transmission zu bestimmen und solchermaßen ein Absorptionsspektrum der undurchsichtigen Probe bei Variation der Wellenlänge der monochromatischen Lichtquelle zu erhalten.

3. Vorrichtung nach Anspruch 1 oder 2, gekennzeichnet weiterhin durch ein optisches System mit zusammenfallenden Brennpunkten, umfassend zwei konvergente optische Systeme (L1, L2), die im Pfad des zweiten Lichtstrahls angeordnet sind, wobei das Mittel zum Unterstützen der streuenden Probe einerseits derart angeordnet ist, um die Probe an dem gemeinsamen Brennpunkt der optischen Systeme (L1, L2) zurechtzulegen, und andererseits Mittel (XY) zum gerasterten Verschieben der Probe relativ zum Brennpunkt aufweist, so daß die mikroskopische Absorptionsverteilung der Probe bestimmt werden kann.

4. Verfahren zur Messung optischer Eigenschaften einer Licht streuenden Probe (20, 21) mit den folgenden Schritten: Aufteilung des Lichtstrahls der monochromatischen Lichtquelle variabler Wellenlänge in einen ersten und einen zweiten Lichtstrahl; Zurechtlegen der Probe in dem Pfad des zweiten Lichtstrahls; Zusammenfassung des Lichts des zweiten Lichtstrahls, der die Probe durchsetzt, mit dem Licht des ersten Lichtstrahls; Umwandlung des zusammengefassten Lichts in ein elektrisches Signal und Bestimmung der Intensität einer Interferenzkomponente des elektrischen Signals; **dadurch gekennzeichnet,** daß das Licht des zweiten Lichtstrahls, welches mit dem ersten Lichtstrahl zusammengefasst wird, durch die Probe hindurchlaufendes Licht ist; daß die Länge des optischen Pfads des ersten Lichtstrahls mit einer Geschwindigkeit verstellt wird, die derart in Beziehung zu der schmalen Bandweite der Lichtquelle gesetzt ist, daß die Interferenzkomponente einen Wechselanteil aufweist, dessen Amplitude durch die Intensität des zweiten, durch die Probe hindurchlaufenden Lichtstrahls bestimmt ist; daß die Wellenlänge des von der Lichtquelle ausgesendeten Lichts verändert wird, und daß die korrespondierende Veränderung der Amplitude der Interferenzkomponente bestimmt wird, um einen Meßwert der spektralen Absorption der Probe zu liefern.

5. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß Licht des zweiten Lichtstrahls, welches durch die Probe hindurchlaufen soll, auf einen Punkt innerhalb der Probe fokussiert wird, die Probe wird relativ zum Brennpunkt gerastert verschoben, und ausschließlich das durch den Brennpunkt hinddurchlaufende Licht wird mit dem Licht des ersten Lichtstrahls zusammengefasst, wobei die mikroskopische Spektralabsorption der Probe bestimmt wird.

Revendications

1. Un appareil pour mesurer les caractéristiques optiques d'un échantillon diffuseur de lumière (20,21) comprenant une source de lumière monochromatique (10) de longueur d'onde variable ; un moyen diviseur de faisceau (BS) pour diviser le faisceau lumineux venant de la source de lumière en un premier et un second faisceaux ; un moyen (M) placé sur le trajet du premier faisceau lumineux pour faire varier la longueur du trajet optique du premier faisceau lumineux ; un moyen pour supporter l'échantillon diffuseur de lumière sur le trajet optique du second faisceau lumineux ; un moyen (HM) pour combiner la lumière du second faisceau lumineux propagée par l'échantillon diffuseur de lumière (20,21) à la lumière du premier faisceau lumineux reçue du moyen de variation (M) et pour projeter la lumière composite résultante dans la même direction ; et un moyen (2) pour recevoir la lumière composite résultante venant du moyen de combinaison (KM) et pour convertir cette lumière composite en un signal électrique, ledit moyen de conversion comprenant un moyen pour détecter l'intensité d'une composante alternative dudit signal électrique ; caractérisé en ce que le moyen de combinaison (HM) est disposé de façon à recevoir la lumière venant du second faisceau lumineux propagée à travers un échantillon opaque (20,21) maintenu par ledit moyen de support, en ce que le moyen de variation est disposé de façon à faire varier la longueur du trajet optique du premier faisceau lumineux à une vitesse prédéterminée dont le rapport avec une bande étroite de la source de lumière monochromatique (10) est tel que la composante alternative dudit signal électrique a une fréquence déterminée par l'interférence entre les premier et second faisceau lumineux, ledit moyen de détection étant disposé de telle sorte que l'intensité de ladite composante alternative est déterminée par l'intensité du second faisceau lumineux propagé à travers l'échantillon et que l'absorption spectrale dans l'échantillon opaque peut être mesurée par la variation de la longueur d'onde de la lumière émise.

2. Un appareil selon la Revendication 1, comprenant également un moyen pour ne détecter que l'intensité du premier faisceau lumineux afin de fournir une intensité de référence et un moyen pour comparer ladite intensité de référence à l'intensité de ladite composante alternative afin de déterminer une extinction intégrale de transmission et d'obtenir ainsi un spectre d'absorption dudit échantillon opaque avec la variation de la longueur d'onde de ladite source de lumière monochromatique.

3. Un appareil selon la Revendication 1 ou 2, comprenant en outre
un système optique cofocal constitué de deux systèmes optiques convergents (L1,L2) placés sur le trajet du second faisceau lumineux, ledit moyen de support de l'échantillon diffuseur de lumière étant disposé de telle sorte que l'échantillon soit situé au point focal commun des systèmes optiques (L1,L2) et comportant un moyen (XY) pour balayer l'échantillon par rapport audit point focal, afin de pouvoir déterminer la distribution d'absorption microscopique dudit échantillon.

4. Un procédé pour mesurer les caractéristiques optiques d'un échantillon diffuseur de lumière, consistant à : diviser le faisceau lumineux venant d'une source de lumière monochromatique de longueur d'onde variable en un premier et un second faisceaux ; placer ledit échantillon sur le trajet du

second faisceau lumineux ; combiner la lumière du second faisceau lumineux propagée par ledit échantillon à la lumière du premier faisceau lumineux, tout en faisant varier la longueur du trajet optique de la lumière du premier faisceau ; convertir ladite lumière combinée en un signal électrique et détecter l'intensité d'une composante d'interférence dudit signal électrique ; caractérisé en ce que la lumière du second faisceau lumineux combinée à la lumière du premier faisceau lumineux est transmise optiquement à travers ledit échantillon ; que la longueur du trajet optique du premier faisceau lumineux est modifiée à une vitesse dont le rapport avec une bande étroite de ladite source de lumière est tel que ladite composante d'interférence a une fréquence alternative dont l'amplitude est déterminée par l'intensité du second faisceau lumineux propagé à travers ledit échantillon ; que la longueur d'onde de la lumière émise par ladite source de lumière est modifiée et que la variation correspondante de l'amplitude de ladite composante d'interférence est détectée pour fournir une mesure de l'absorption spectrale dudit échantillon.

5. Un procédé selon la Revendication 4, dans lequel la lumière du second faisceau à transmettre à travers ledit échantillon est focalisée sur un point dudit échantillon, ledit échantillon est balayé par rapport au point focal, et seule la lumière transmise à partir dudit point focal est combinée à la lumière du premier faisceau lumineux, ce qui permet de déterminer l'absorption spectrale microscopique dudit échantillon.

FIG. 1

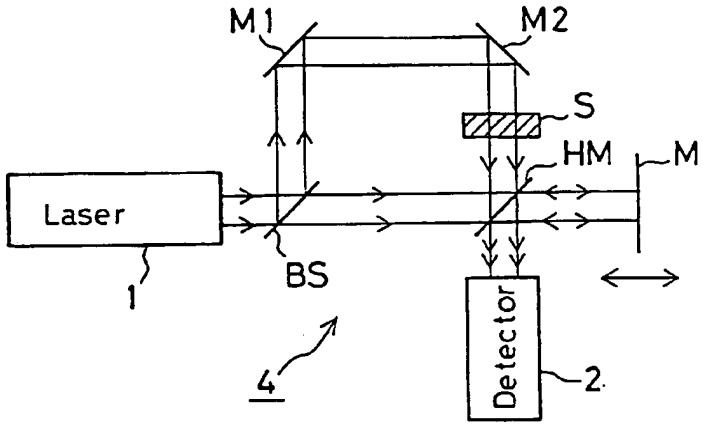

M1
M2
S
HM
M
Laser
BS
1
4
Detector
2

FIG. 2

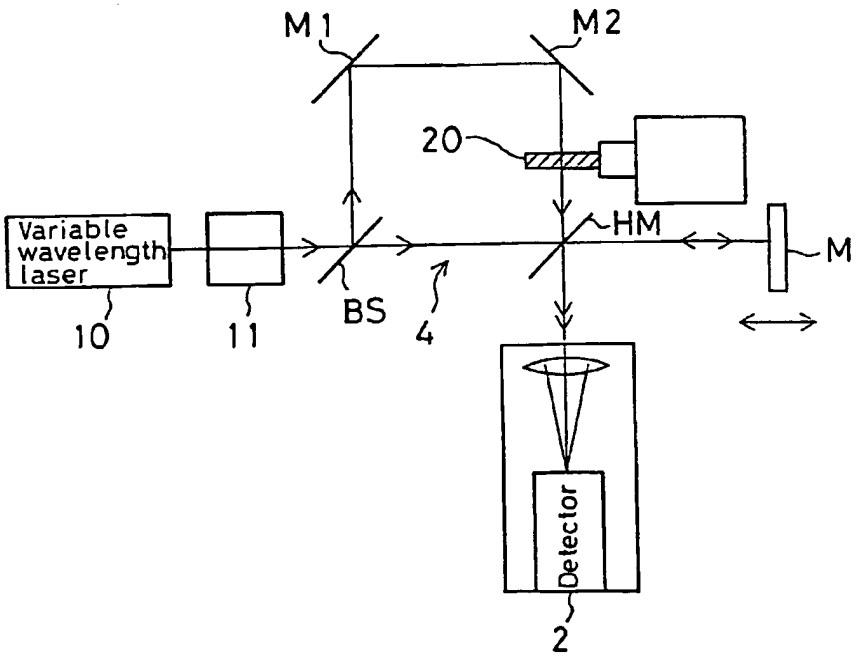

M1
M2
20
HM
M
Variable wavelength laser
BS
10
11
4
Detector
2

FIG. 3

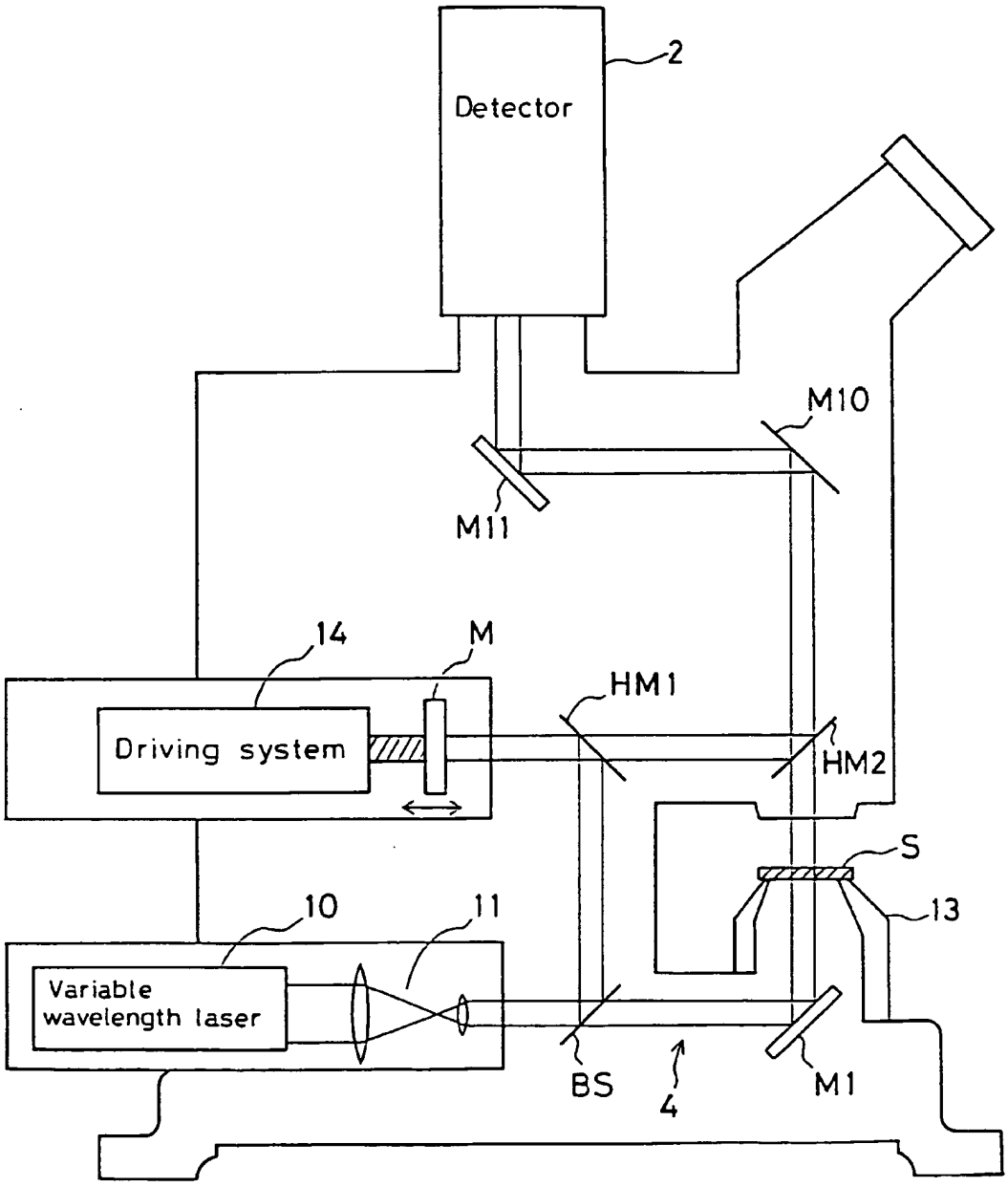

Detector
2
M10
M11
14
M
HM1
Driving system
HM2
S
13
10
11
Variable
wavelength laser
BS
4
M1

FIG. 4

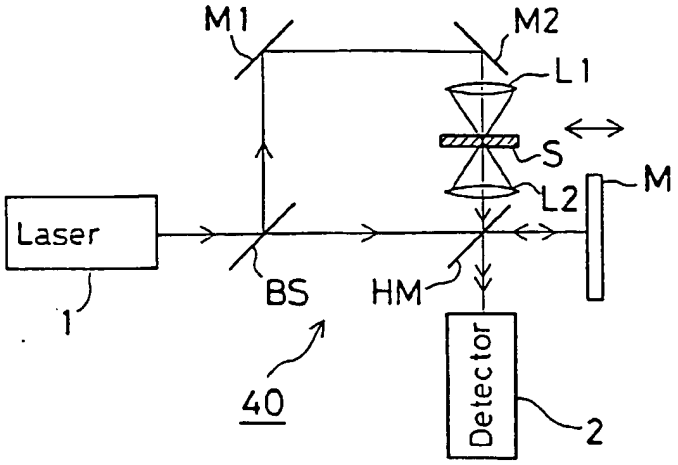

M1
M2
L1
S
L2
M
Laser
BS
HM
1
40
Detector
2

FIG. 5

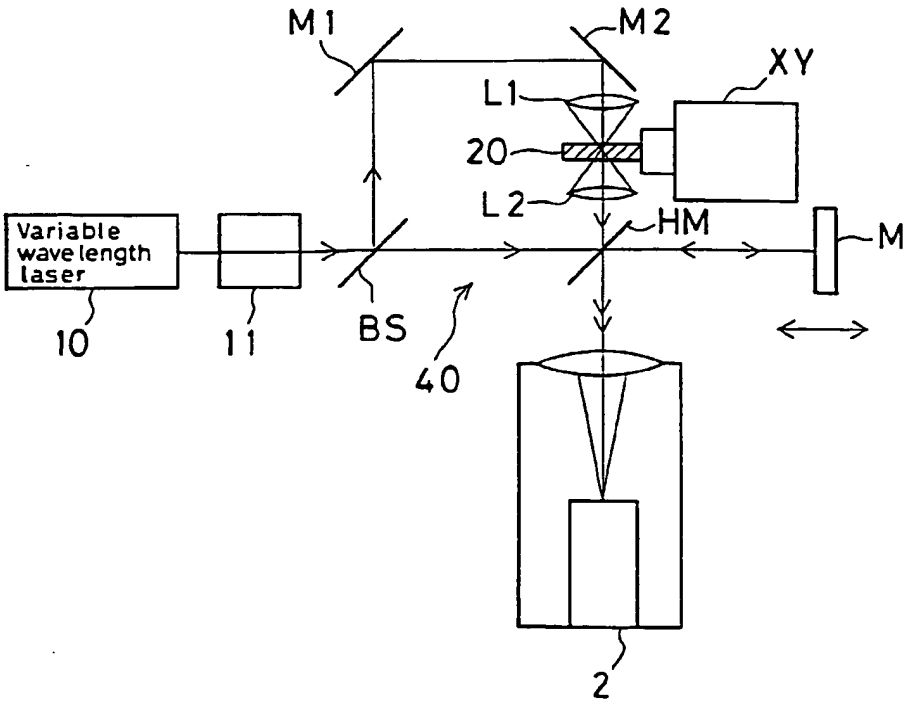

M1
M2
XY
L1
20
L2
HM
M
Variable wavelength laser
10
11
BS
40
2

FIG. 6

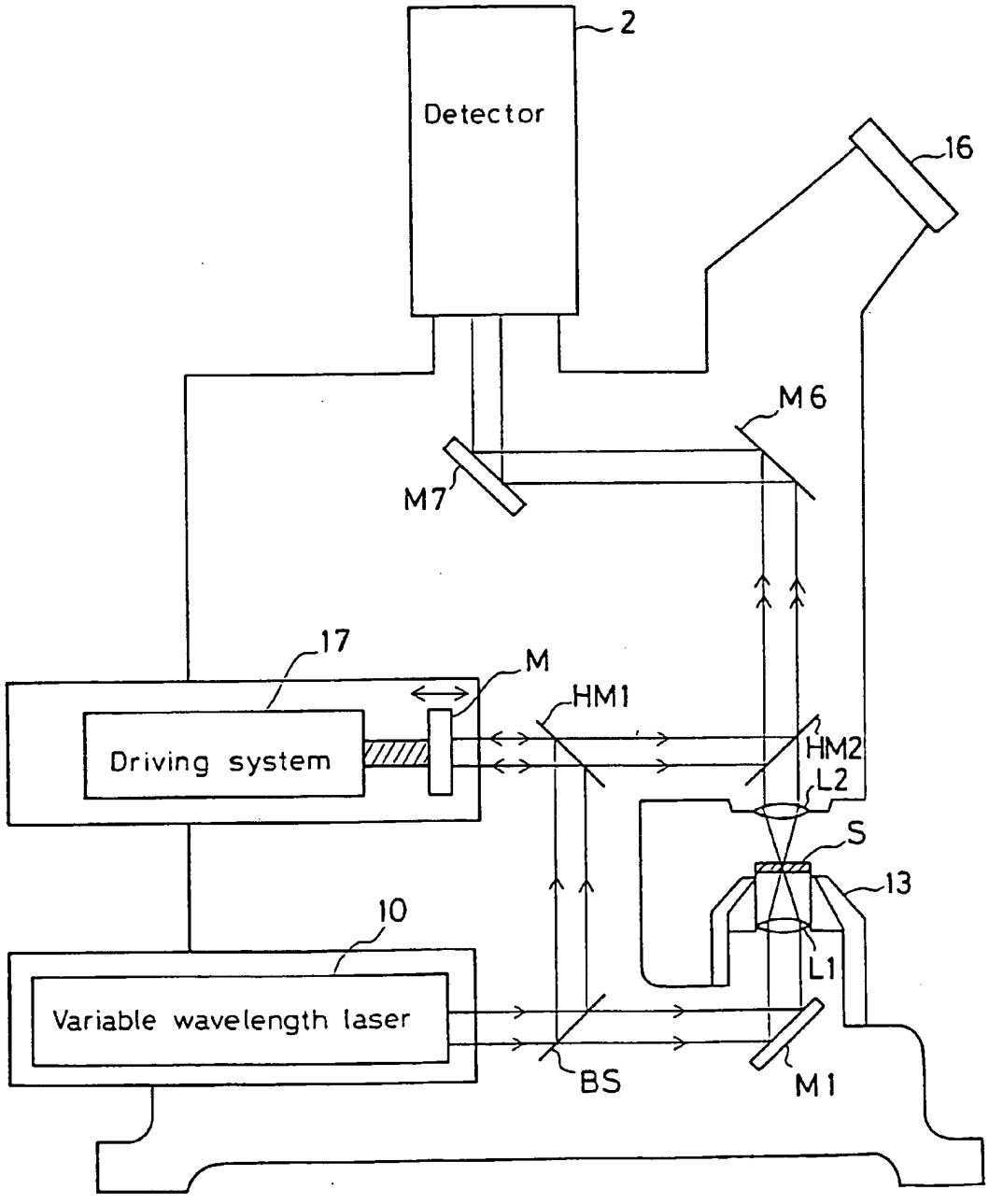

2
Detector
16
M6
M7
17
M
HM1
Driving system
HM2
L2
S
13
10
L1
Variable wavelength laser
BS
M1

FIG. 7

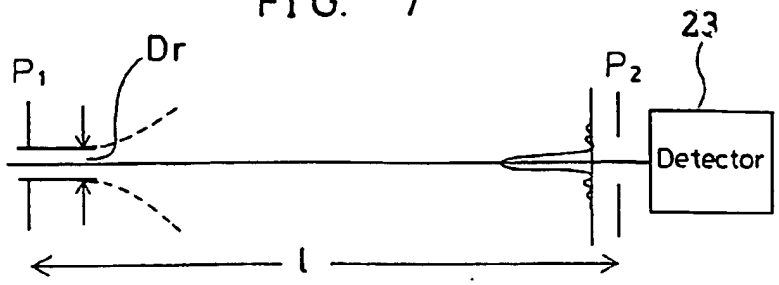


FIG. 8

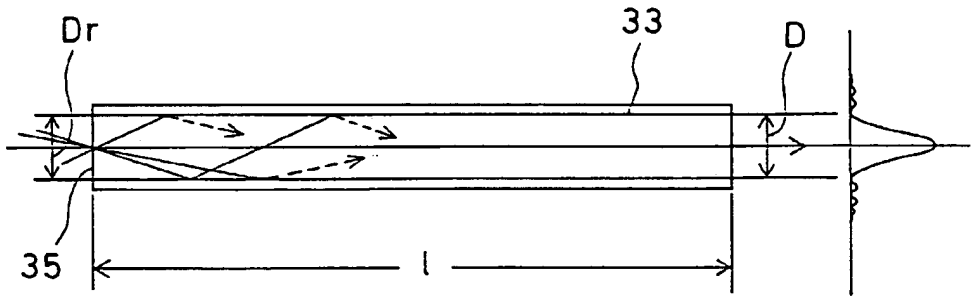


FIG. 9

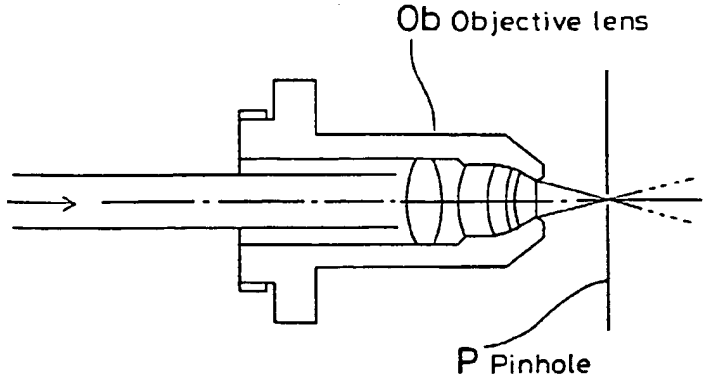


FIG. 10

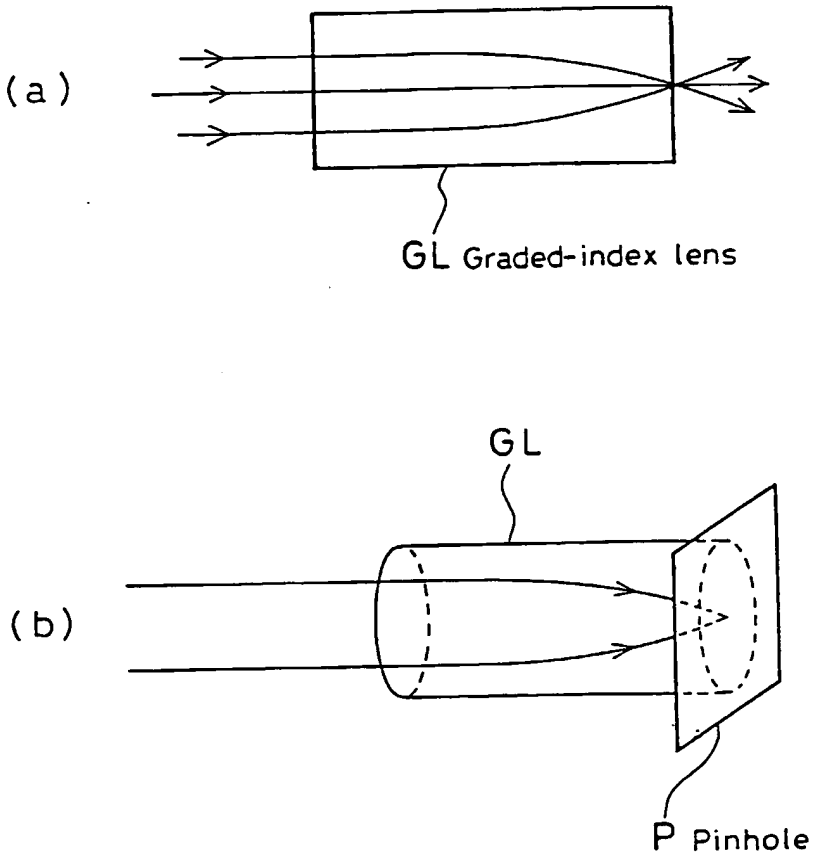


FIG. 11

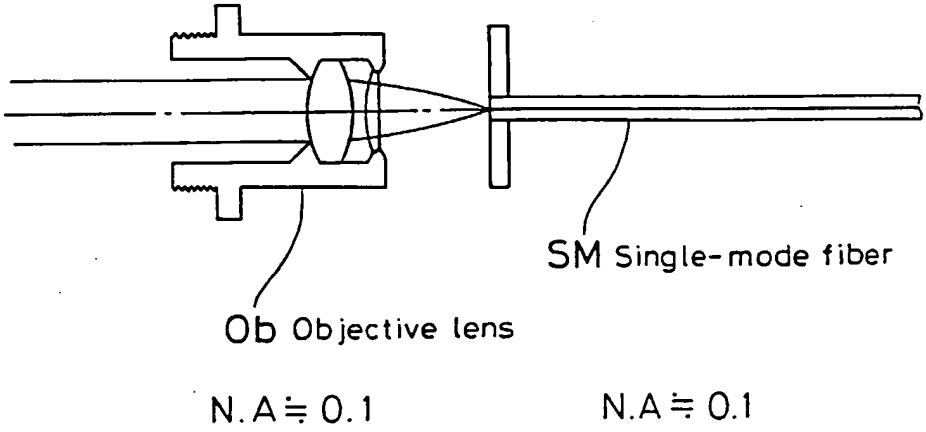


FIG. 12

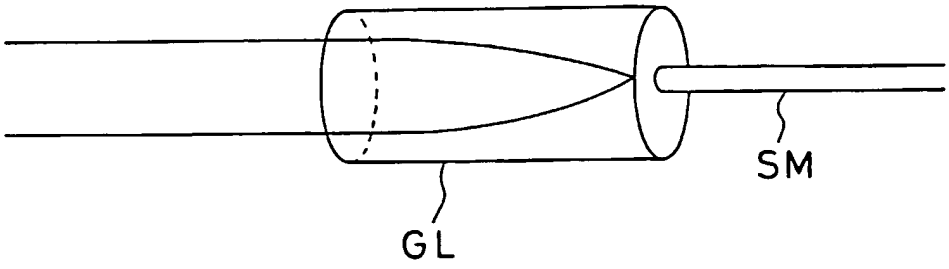


FIG. 13

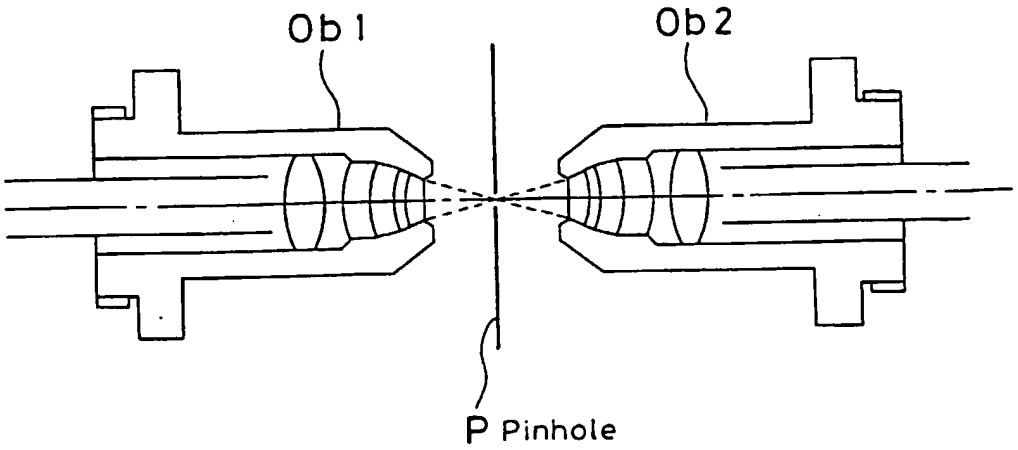


FIG. 14

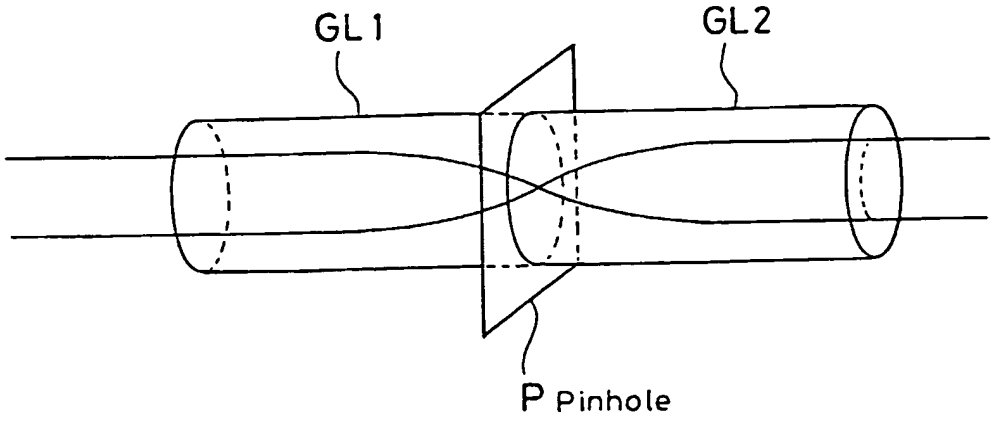


FIG. 15

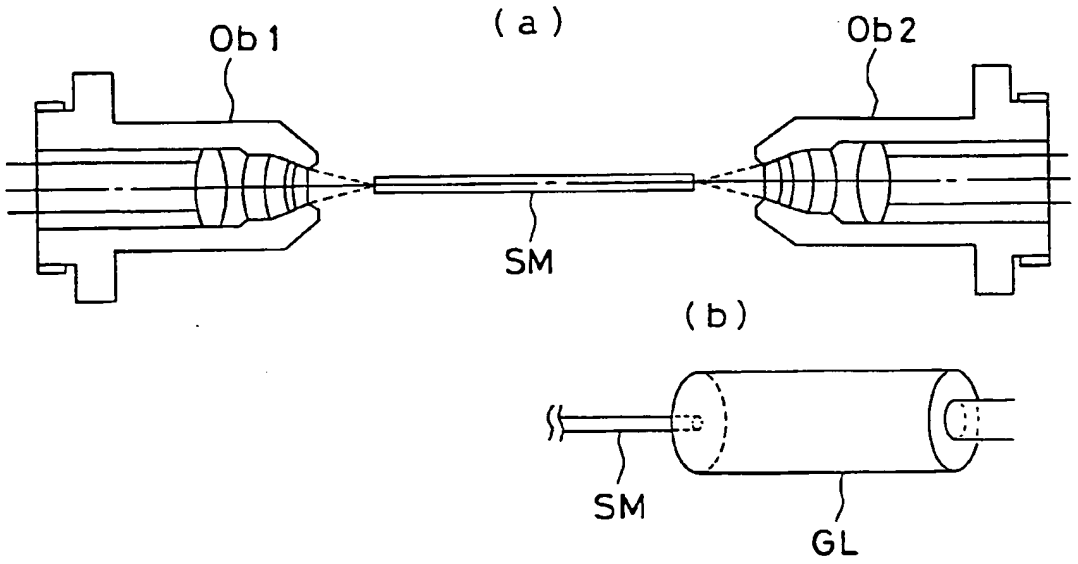


FIG. 16

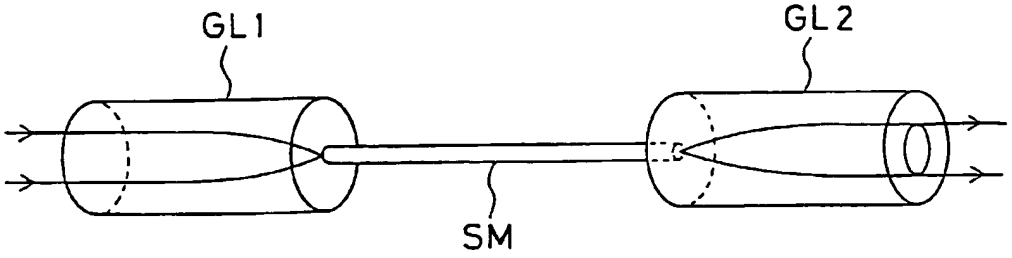
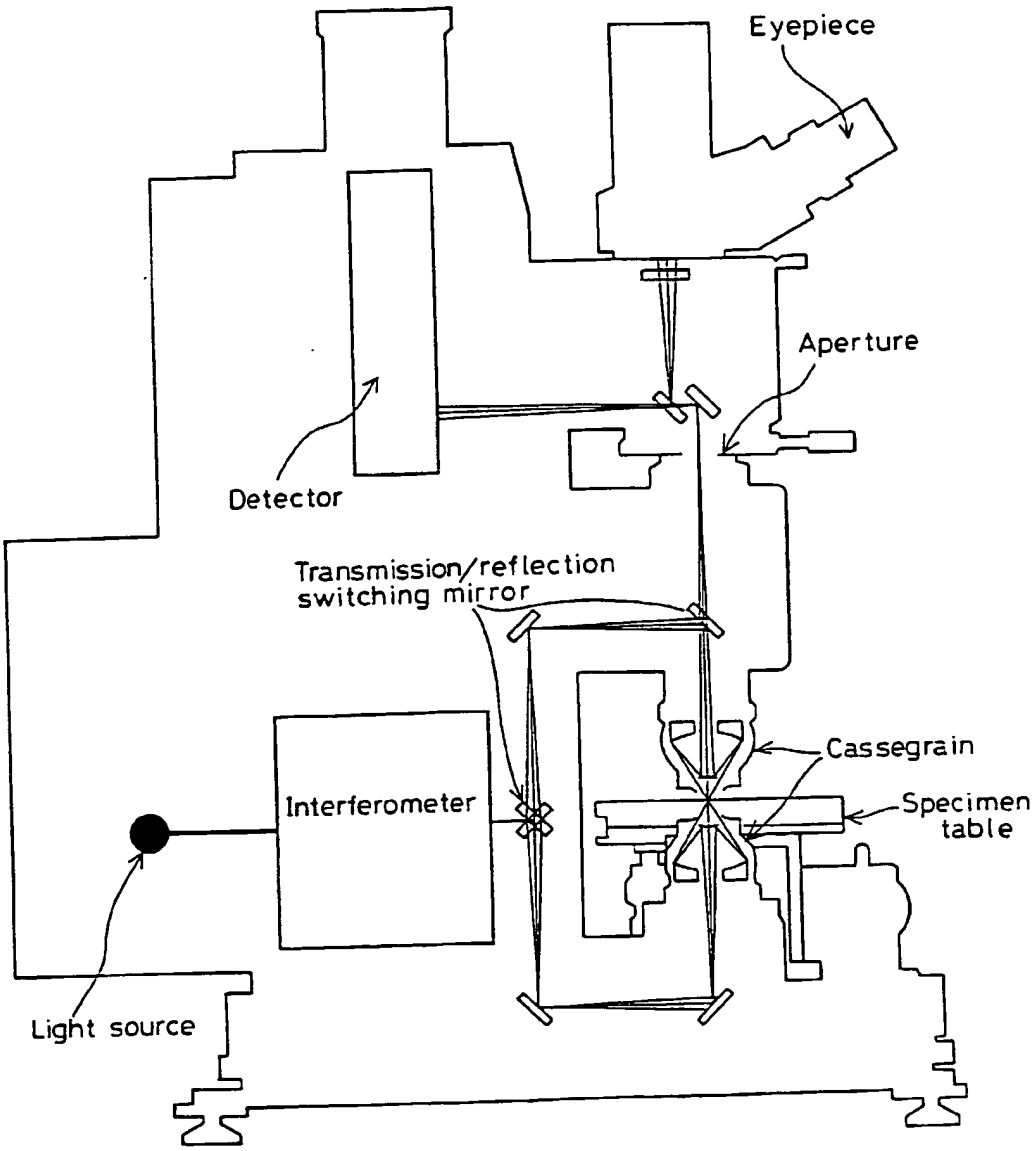


FIG. 17


Eyepiece
Aperture
Detector
Transmission/reflection
switching mirror
Cassegrain
Interferometer
Specimen
table
Light source

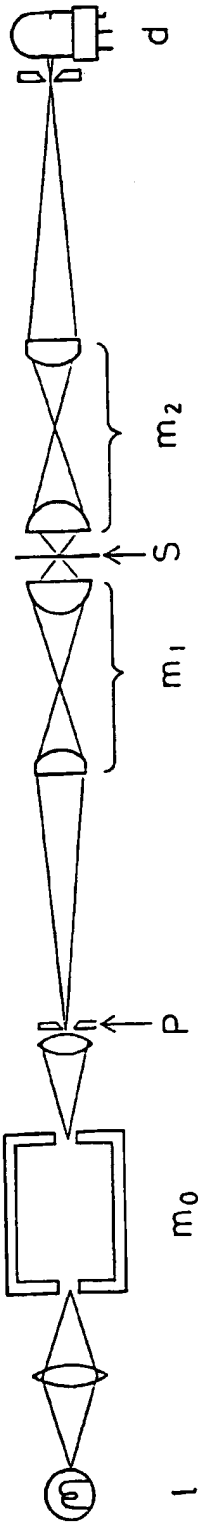


FIG. 18